Ida Pan

Cur file 5200

Copy sent P. Wilson Route
Goodman
Cannibear
Farnan 2c.

STUDY PLAN

A PILOT CONTROL PROJECT AERIALLY APPLYING A NUCLEOPOLYHEDROSIS VIRUS AND *BACILLUS THURINGIENSIS* TO DOUGLAS-FIR TUSSOCK MOTH POPULATIONS IN IDAHO 1974

Prepared by _____ March 13, 1974
Chairman, Interagency Douglas-fir Date
Tussock Moth Pilot Project Task Force

Reviewed and Approved:

IDAHO PANHANDLE N. F.

X = ACTION	√ = INFO
FS	OPERATIONS
DEPUTY	Law
RESOURCE	Res
Wtrsh	Pers
Fire	Adm Sv
Post Sale	Mgt A
Pre Sale	B & F
Rec/L	Mail File
Rg/WL	
Prog A	Conbear
Mnrls	

MAY 20 1974

PLANNER	SERVICES
Coord Use	Plans
Fish Biol	Env
Geol	Commu
T.M. Plnnr	
Illustr	

SEND COPIES TO:
SANDPOINT PLAN
SANDPOINT ENGR
ST. MARIES PLAN
ST. MARIES ENGR

_____ March 13, 1974
for Chief, Division of State and Date
Private Forestry, Region 1

_____ 3/13/74
for Regional Forester, Region 1 Date

Robert F. Farnum 3/28/74
for Director, Pacific Northwest Date
Forest & Range Experiment Station

_____ 5/3/74
Director, Forest Pest Control Date
Washington Office

U.S. DEPARTMENT OF AGRICULTURE - FOREST SERVICE
Division of State and Private Forestry
Northern Region Missoula, Montana

TABLE OF CONTENTS

INTRODUCTION

The Douglas-fir tussock moth, *Orgyia pseudotsugata* McD., periodically reaches epidemic levels in the Northwest. Detailed histories of outbreaks in the Northern Region, Oregon, and California are discussed by Wickman et al. (1973) and Tunnock (1973). Past outbreaks have usually persisted for 2 to 4 years at which time they die out due primarily to epizootics of a nucleopolyhedrosis virus.

With the advent of DDT in the mid-1940's came the first promising insecticidal treatment for broad-scale Douglas-fir tussock moth control. In 1947 over 400,000 acres of tussock moth infested forests in Idaho were aerially sprayed with 1 pound of DDT in 1 gallon of fuel oil per acre. Another 120,000 acres of tussock moth infested forests were sprayed with DDT in Idaho in 1965 (Tunnock 1973).

As the undesirable properties of large-scale DDT spraying became known, the Forest Service discontinued spraying with this insecticide. DDT spraying was terminated in Region 1 in 1965. Since then work has been conducted to develop environmentally acceptable materials for Douglas-fir tussock moth control. At least 12 chemical insecticides and two microbial agents have been either laboratory or laboratory and field tested against the tussock moth (Anon. 1973). Two microbials, a nucleopolyhedrosis virus and the bacterium *Bacillus thuringiensis* Berliner, have shown the most promise of the materials field tested to date.

The most recent field tests of these materials against the Douglas-fir tussock moth were conducted in eastern Oregon in 1973. Various treatments were applied by helicopter to 20-acre field plots. Preliminary results showed that larval populations were reduced from 74.5 to 99.9 percent depending on the formulation (Thompson and Markin 1973). As a result of these successful field tests the next step in the developmental process of these materials is a pilot control project.

The current tussock moth outbreak in northern Idaho is believed to provide the elements needed for a definitive pilot project with virus and bacteria. The elements as described by Stelzer and Neisses (1973) are:

1. Relatively high tussock moth larval densities.

2. A population in the release phase of the outbreak cycle (as defined by Wickman et al. 1973).

3. A population with a very low level of natural virus associated with the overwintering eggs.

OBJECTIVES

The objective of this project is to separately evaluate the effectiveness of an aerial application of Dipel (*Bacillus thuringiensis* or *B.t.*) and a nucleopolyhedrosis virus in reducing Douglas-fir tussock moth larval populations when applied under normal operational conditions.

Secondary objectives include:

1. Determine the extent of foliage protection achieved by the treatments.

2. Investigate the correlation of spray deposit with larval mortalities.

3. Determine the incidence of parasitism of the Douglas-fir tussock moth under the limitations of the sampling schedule.

METHODS

Treatments

The pilot control project will be conducted at two locations, Coeur d' Alene Mountain south of Coeur d'Alene, Idaho, on the Idaho Panhandle National Forests (fig. 1) and Lookout Butte, south of Lowell, Idaho, on the Nezperce National Forest (fig. 2). Both materials will be applied at each location.

An aerial application of virus and bacteria, formulated as shown in Table 1, will be applied to both areas at the rate of 2 gallons per acre.

Table 1.--Composition of proposed spray formulations to be applied per acre

Active ingredients	Adjuvants (gallon)	NaOH (grams)	B.S.F.[1/] (grams)	Sun screen (pound)	Water (gallon)
Douglas-fir tussock moth virus (100 billion polyhedra)	C.I.B.[2/] (.5)	26.4	7.6	IMC 90-001 (1.0)	1.5
Bacillus thuringiensis (1 pound)	C.I.B. (.5)	--	7.6	--	1.5

1/ Brilliant sulfur yellow dye.

2/ Cargills insecticide base (molasses).

Fig. 1
Coeur d'Alene Mountain infestation area,
site of proposed spray plots.

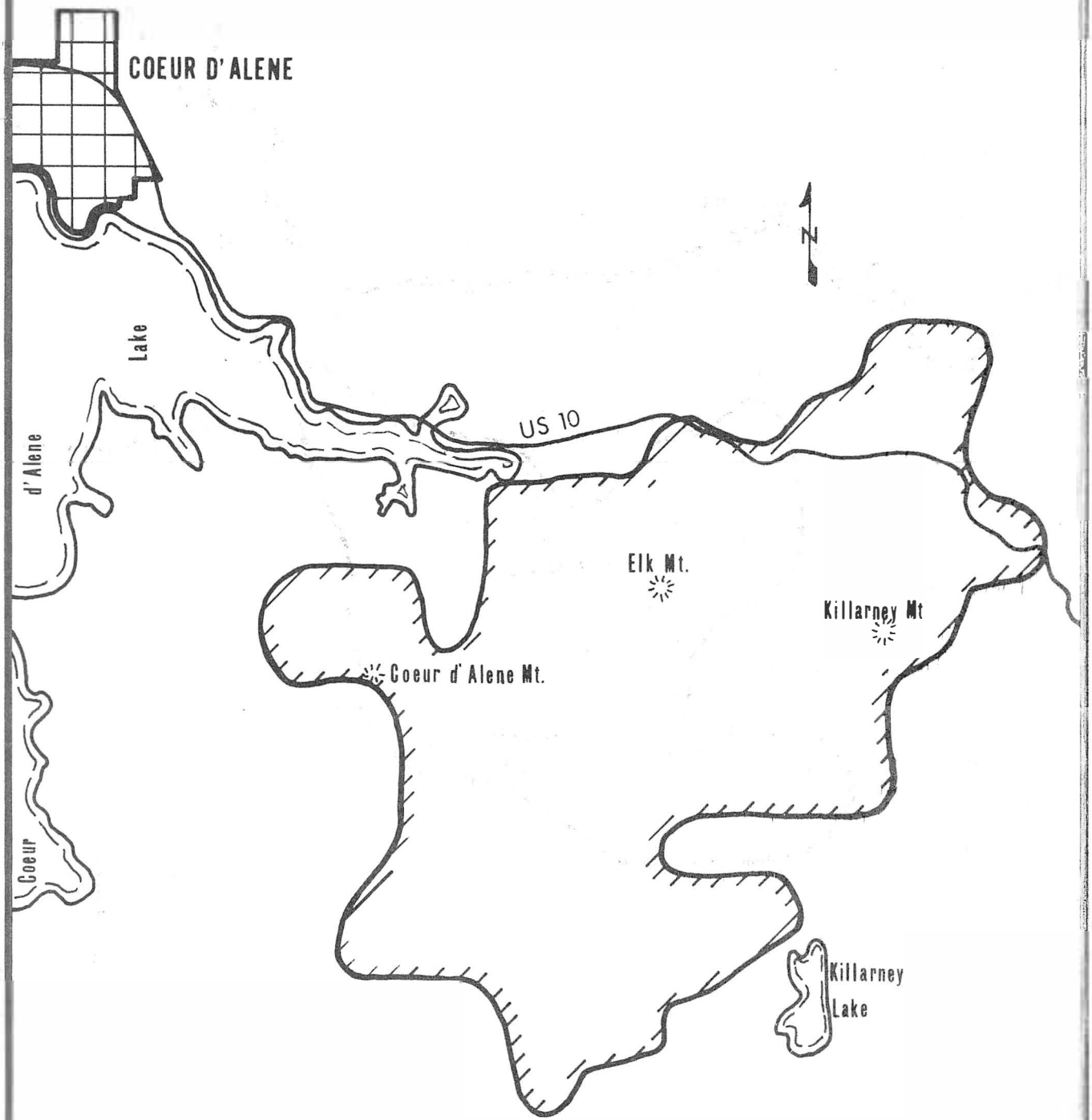
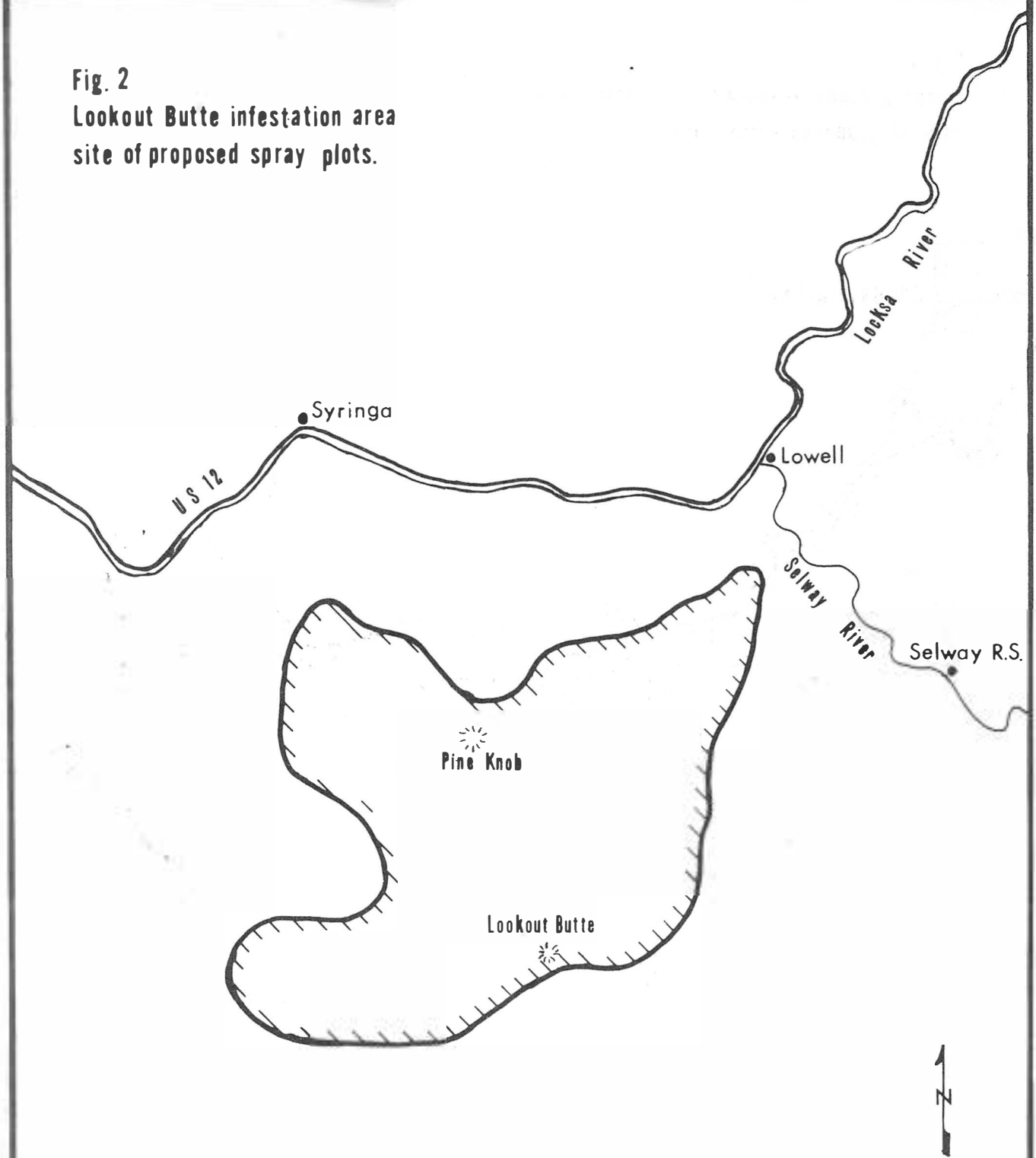


Fig. 2
Lookout Butte infestation area
site of proposed spray plots.



A portable pH meter will be used to monitor the pH of the spray suspension during the mixing operation. The pH should remain near 6.6 ± 0.3. Since the pH of the molasses solution may vary from batch to batch, additional amounts of NaOH pellets may be required to stabilize the pH at the desired level.

The two areas will each be divided into nine plots (three virus, three bacteria, and three controls). Plots will not necessarily be the same size or shape. Natural boundaries, i.e., creeks, ridges, type changes, burns, clearcuts, etc., will dictate their size. A minimum of near 1,000 acres and a maximum of about 5,000 acres per plot have been set as guidelines.

Within each plot an 800-acre area will be identified as a sampling zone. All sample trees for measuring tussock moth population levels will be located within this area.

Enough virus is available to treat only 6,500 to 7,500 acres. Hence, the six virus plots will be about 1,000 acres in size.

Criteria for Test Plot Selection

Plots must have good accessibility and somewhat natural boundaries. Tussock moth population levels and current defoliation should be more or less uniform among the plots. The sampling portion of the plots will be located a sufficient distance from the next plot to minimize contamination by spray drift. Distances will vary with the terrain and wind patterns.

Natural Virus Level Determination

Egg masses were collected from 26 sections of land within the proposed Coeur d'Alene Mountain treatment area and from 22 sections within the Lookout Butte area as a part of the 1973 egg mass survey. These were used for the virus level determination. Five egg masses per section, if that many were available, were used. Egg masses were labeled as to collection site and placed in refrigeration at approximately 35° F.

About February 1, these masses were bisected and one-half of each mass sent to the Insect Disease Project (PNW-2203) and the other half kept in Missoula. Eggs are now being hatched and 20 to 30 larvae per egg mass are being reared on artificial diet in a single Petri dish. Fourteen days after hatching, survival of larvae from each egg mass will be determined. Dead larvae will be diagnosed for cause of death. The virus level in the overwintering population will thus be determined by early March. Egg parasitism and percent egg hatch will also be determined at this time.

If an average of 5 percent or more of the larvae from an area are infected with the naturally occurring virus, that area will be deleted as a test site. If the virus level is between 1 and 5 percent, other

factors such as tussock moth population density will have to be evaluated before the area can be accepted or rejected as a test location. If the natural virus level is less than 1 percent, the area can be considered usable in this regard.

If virus levels are too high in the proposed areas, alternate test sites will be searched for in Idaho, Washington, or Oregon. Possible alternative test sites will be identified by mid-March following the laboratory virus level determinations.

Sampling Design[1/]

An analysis of variance was made from the prespray Douglas-fir tussock moth data from a test of Sevin-4-oil conducted by the Idaho Department of Public Lands during 1973 to determine the most desirable number of branches per tree, trees per cluster, and clusters per area to estimate a sample mean (Table 2). A nested design was chosen to measure the variability between areas, clusters, trees, and branches. The trees were to be selected in clusters of 10, although often the size of area enclosing the 10 trees was quite variable.

Table 2.--Analysis of variance for branches, trees, clusters, and areas

Source	Degree of freedom	Sum of squares	Mean squares
Total	639	2,497,470	3,908.40
Areas	3	779,202	259,734.00
Clusters	12	294,276	24,523.00
Trees	144	729,473	5,065.79
Branches	480	694,518	1,446.91

An estimate of the sample variance using different combinations of branches per tree, trees per cluster, and clusters per area can be used to determine optimum sample size for the 1974 tussock moth pilot projects.

The estimated sample variance can be calculated in the following formula (Snedecor 1956):

1/ The experimental design was chosen by consultation with Dr. Donald V. Sisson, biological statistician, Utah State University, Logan, Utah, and Dr. Michael Marsden, Northern Fire Laboratory, Missoula, Montana.

$$\sigma^2_x = \frac{\sigma^2_b}{btc} + \frac{\sigma^2_{bt}}{tc} + \frac{\sigma^2_{btc}}{c}$$

Where σ^2_x = estimated variance of branches, trees, clusters

σ^2_b = mean square value for branches

σ^2_{bt} = $\frac{\text{mean square for trees - mean square for branches}}{\text{number of branches per tree taken}}$

σ^2_{btc} = $\frac{\text{mean square for clusters - mean square for trees}}{\text{number of branches x number of trees}}$

b = number of branches per tree
t = number of trees per cluster
c = number of clusters per area

The predicted variance (σ^2_x) using different combinations of branches, trees, and clusters is shown in Table 3. The mean prespray population in the 1973 Idaho test was 64.95 larvae per 1,000 square inches of foliage. The values of σ^2_x is the estimated variance of that mean.

Table 3.--Estimated variance for various combinations of branches, trees, and clusters

4 branches, 10 trees, 4 clusters (40 trees/area)

$$\sigma^2_x = \frac{1,446.91}{4x10x4} + \frac{904.72}{10x4} + \frac{486.4}{4} = 153.26^{1/}$$

2 branches, 10 trees, 4 clusters (40 trees/area)

$$\sigma^2_x = \frac{1,446.91}{2x10x4} + \frac{904.72}{10x4} + \frac{486.4}{4} = 162.30$$

2 branches, 10 trees, 8 clusters (80 trees/area)

$$\sigma^2_x = \frac{1,446.9}{2x10x8} + \frac{904.72}{10x8} + \frac{486.4}{8} = 81.20$$

2 branches, 8 trees, 10 clusters (80 trees/area)

$$\sigma^2_x = \frac{1,446.9}{2x8x10} + \frac{904.72}{8x10} + \frac{486.4}{10} = 68.99$$

2 branches, 5 trees, 16 clusters (80 trees/area)

$$\sigma^2_x = \frac{1,446.9}{2x5x16} + \frac{904.72}{5x16} + \frac{486.4}{16} = 50.75^{2/}$$

1/ Combination of branches, trees, and clusters used in the 1973 Idaho test.

2/ Combination of branches, trees, clusters chosen for 1974 tests.

Little is gained by taking four branches per tree compared to taking two (Table 3). More trees, rather than branches, per area will reduce the variance estimates to a greater degree. There is also an advantage in taking only two branches so that subsequent postspray sampling would not deplete desirable branch samples as rapidly as four branches per postspray sample. Postspray samples may be biased if a tree does not contain an excess of good sample branches.

The analysis also points out that more clusters per area and fewer trees per cluster would decrease the variance without increasing the number of sample trees. There is, however, a point at which a cluster should contain enough trees to assure that negative mortality for the cluster would not occur. Negative mortality may become critical if regression is used to correlate deposit with mortality.

Two other important points favoring fewer trees per cluster and more clusters are:

1. It is more difficult to obtain 8 to 10 trees in a cluster than 4 to 6 trees because of the availability of suitable sample trees in the cluster area.

2. Fewer trees per cluster would have a greater chance to receive uniform deposit because of spray coverage variability. A small cluster would be more likely to be sprayed by a single swath than a large cluster covering more area.

Based on the estimated variances and what is feasible manpowerwise, the selected sample design is to cluster samples, taking two branches per tree, five trees per cluster, and 16 clusters per area for a total of 80 trees per area. Clusters will be located at least 5 chains apart and should not occupy more than 1 acre of ground. Clusters will be located throughout the spray area. If it is impossible to cluster sample because five trees cannot be found on 1 acre, or 16 clusters cannot be located in a plot, single tree samples will be used.

The two project areas (Coeur d'Alene Mountain and Lookout Butte) will be sampled identically. If the single tree method is necessary in one area, it will be used in both. The area judged most difficult to cluster sample will be laid out first to determine if the cluster method is feasible or if the sample design will be based on single trees.

Using the same data from the 1973 Idaho tests, an analysis of variance was made assuming that the trees were not clustered in order to select a suitable number of sample trees if the single tree sampling method is needed (Table 4).

Table 4.--Analysis of variance for areas, trees, and branches

Source	Degree of freedom	Summary of squares	Mean squares
Total	639	2,497,470.0	3,908.40
Areas	3	779,202.0	259,734.00
Trees	156	1,023,750.0	6,562.49
Branches	480	694,518.0	1,446.91

Following the procedure described previously, a two-way table was constructed showing various combinations of branches and trees to estimate a variance for the population mean at 64.95 larvae per 1,000 square inches of foliage (Table 5). From this a sample size of 80 trees/plot, two branches/tree was selected if the single tree method is used.

Table 5.--Estimate of variances for different combinations of branches and trees per area

No. trees	Branches per tree 1	2	3	4	5
30				54.680	52.270
35			50.319	46.870	44.800 1/
40		50.064	44.030	41.014 2/	39.206
45		44.496	39.138	36.458	
50	54.516	40.047	35.217	32.811	
55	49.560	36.406	32.022		
60	45.430	33.372			
65	41.935	30.805			
70	38.939	28.604			
75	36.343	26.697			
80	34.072	25.029 3/			

1/ Sample below line shows an estimated variance which would be less than the 1973 Idaho tests data.

2/ Variance for the number of branches and trees for sample data from the 1973 Idaho test.

3/ Sample size chosen for 1974 tests and estimated variance.

Description of Sample Trees

Sample trees are defined as 30 to 50 feet tall Douglas-fir or grand fir. This height is used because of equipment limitations in sampling taller trees. Sample trees should be somewhat open grown and not shielded by larger trees. They must have sufficient foliage to support high tussock moth populations.

Timing of Application and Development Sampling

Spraying will begin when 50 percent of the larvae have reached the second instar. This will probably be in late June. Timing of application will be determined by:

1. Tagging 40 egg masses per plot prior to ecosion and examining them two or three times a week for hatch. Newly hatched larvae cluster on the mass for a day or two before they disperse. After 50 percent of the egg masses have hatched, branch samples will be collected for instar determination.

2. Two midcrown branches from 20 trees per plot will be collected every other day when 50 percent of the egg masses have hatched. These will not come from population sampling trees. Development sampling will give field crews experience using pole pruners and processing samples. Samples will be taken daily from each unit when 20 percent of the larvae are second instar. Larvae will be placed in alcohol and taken to the laboratory for instar determination by head capsule measurement.

Population Sampling

Douglas-fir tussock moth larval populations will be sampled within 48 hours prior to spraying and again following spraying at 7, 21, and 35 days, and shortly before pupation. Control plots will be sampled at the same time intervals. The population sample will be taken by extending a 24-foot telescopic pole pruner with catch basket attached into the midcrown portion of the sample tree and clipping approximately the distal 18 inches of two branches from opposite sides of each tree sample in such a way that they fall into the basket. Two branches will be collected per sample tree each sampling period. Branch samples and all contents of the basket will be carefully placed on canvas tarps for larval counting and branch measurement. The following information will be recorded at each tree:

1. Date
2. Treatment area
3. Tree number
4. Tree species
5. Branch length
6. Branch width
7. Number of tussock moth larvae
8. Crew leader

The basic population measurement unit will be tussock moth larvae per 1,000 square inches of foliage.

A survey of the egg mass population within the plots will be conducted during fall 1974. This will be to determine if the treatment effects

carry over to the overwintering population; i.e., if there are proportionately fewer egg masses in treated areas compared to untreated areas.

Sampling crews will be given special instructions and training in the field before the actual sampling is started.

Laboratory Activities

A field laboratory will be operated at both project areas. The main functions of this facility are:

1. Serve as project headquarters
2. Provide suitable conditions for laboratory rearing of larvae
3. Provide a place for diagnostic work
4. Provide accommodations for preliminary data examination

If available, 10 larvae will be collected for laboratory rearing from each sample tree during each sampling period. These will be collected by the field crews and placed separately in Petri dishes containing artificial media. The main objective is to diagnose dead larvae to determine the cause of mortality (virus, bacteria, parasites, unknown) on the 14th day after collection. In addition, any measurable differences in the parasite complex present at each sampling period will be determined.

Mixing

Virus.--The available virus preparation is unsuitable for mixing and aerial application due to the presence of body parts and hairs remaining from its preparation. This "dirty" stock material must be cleaned up a day or two (no more than 4) before mixing of the final spray formulations. Cleanup consists of mixing the freeze-dried "dirty" virus in 0.02 molar solution of Trizma 7.1 buffer; then, using a small capacity mixing tank with some form of recirculation, progressively filtering the liquid through 50-, 100-, and 150-mesh screen if a Bell 205A or Bell 206 helicopter is to be used. If a Bell 47G is used, a final filtering through a 200-mesh screen should be included. More details on virus cleanup are included in Appendix I.

Mixing sequence for virus is:

1. Fill mixing tank with water and if cold, heat or allow to stand until temperature exceeds 60° F.

2. Add brilliant sulfur yellow dye (BSF), mix 5 minutes.

3. Add sunscreen, using suction type system and mix for 20 minutes.

4. Add molasses by slowly pouring into mixture. Molasses temperature should be 70° to 80° F. for good flow ability.

5. Check pH and adjust by adding NaOH to a pH of 6.6 to 7.2 and mix thoroughly.

6. If the carrier formulation is mixed the day before use, it can be stored overnight and the virus added the next morning. Before adding the virus, the pH should again be checked and the formulation mixed again for 10 minutes.

7. The virus solution should be directly added to the carrier solution and mixed for 10 minutes before loading.

8. In case of an emergency, unused finished virus spray can be kept 24 hours if constant check is made of its pH to see that it does not exceed 7.5, but whenever possible virus should be added just before loading.

Dipel.--Steps in mixing Dipel are:

1. Fill mixing tank with water and if cold, allow to stand or heat to 60° F.

2. Add dye and mix 5 minutes.

3. Add Dipel, using suction type system and mix for 20 minutes.

4. Add molasses and mix for 20 minutes. For easy pouring, molasses should be 70° to 80° F.

Filtering.--With both Dipel and virus, mixing should be done with a recirculating system (besides mechanical agitation) and both must be filtered. If a Bell 205A or Bell 206 is to apply the material, a 60-mesh screen should be used both in mixing and in the helicopter's in-line filter. No nozzle screens are needed. If a Bell 47G is used, the mixing tank filter should be 100-mesh and the helicopter's in-line screen and nozzle screen should be 50-mesh. The helicopter in-line screen should be checked and cleaned after each load, and the nozzle screen after every other load when virus is used.

Handling.--It must always be remembered that the agents being used are living organisms and not inert chemicals and are very likely to be killed by conditions that would have no effect on chemical insecticides. Microbials, therefore, need much more care in handling and preparation. The water to be used should be checked to be certain it contains no contaminants that could affect the microbials, such as chlorine found in most cities' water supplies. Before using, all mixing and spray equipment should be thoroughly cleaned and checked to be certain they contain no detergents, chemicals, or solvents that could affect the microbials. Temperature is also very critical, and Dipel and freeze-dried virus should always be shipped and stored at termperatures less than 90° F. Both materials must also be kept completely dry until mixing. A very critical watch must also be kept during mixing and

and agitation in the helicopter to see that the temperature does not rise above 100° F. for more than a few minutes.

Spray Application (Equipment and Timing)

Spray will be applied with helicopters. The spray system will have circulation or agitation in the tank and be capable of maintaining pressures of at least 50 pounds per square inch. Boom length and nozzle configuration will be such to allow a 2-gallon-per-acre treatment with a droplet size of 150 to 200 m.m.d. Swath width at tree top height will be 50 to 100 feet at a release height of 50 feet above the tree tops, depending on helicopter type.

Spraying will be done during the early morning hours when the air is cool and still. Spraying will be terminated when winds exceed 6 miles per hour at ground level in clearings or temperatures are over 65 degrees.

An attempt will be made to spray one plot (one-third of a treatment) per day per area. The sampling zone will receive treatment priority. The remainder of the plot will be treated within 48 hours of the sampling zone.

Collection and Assay of Field Samples

One 20 ml. spray sample will be collected at the spray system nozzle immediately after application of each tank load of the suspension. Containers will be supplied. Each container shall be labeled and immediately stored in an ice chest. Biological assay of the sample and polyhedron counts will be performed at the Pacific Northwest Forest and Range Experiment Station laboratory in Corvallis, Oregon, as described by Martignoni, 1970. If there is a possibility that the spray tanks might be completely emptied in flight, then one sample should be collected at the nozzles before each application to insure that at least one set of samples will be available for study. The most important samples are those collected after the application. If available, these samples will be used for bioassay, whereas the set of samples collected before that particular application will be discarded. This redundancy is indicated in order to secure at least one set of samples per application.

A predetermined number of larvae will be inoculated with a sample from each helicopter load with a micro injector. The objective of this is to compare virulence of material going into the spray tanks with that coming out. A malfunction in a recirculation system could create sufficient heat to de-activate the pathogens.

Spray Deposit Sampling

The same trees will be sampled for insect mortality and spray deposit. The basic deposit sampling unit will be four 10- to 15-inch branches

taken from the midcrown of each sample tree, two aluminum plates 6 by 6 inches, and one white Kromekote card. The plates and white card will be positioned at ground level in the nearest open area adjacent to each sample tree. The plates and cards will be placed in the field just prior to spraying. Cards and plates will be collected immediately after spray application. Aluminum plates will be placed face to face (deposit sides together) and stored in slotted boxes for transportation. The four foliage samples will be collected from each cardinal direction of sample trees and placed in brown paper bags. Tree number, sample direction (north, south, east, west) and time of collection will be recorded on each bag.

Environmental Monitoring

Both materials to be tested have been determined to be very host specific. Dipel affects a number of lepidopterous larvae, and the virus affects only a few species of tussock moth.

Dipel is considered so safe to man that it is exempt from tolerance limits. That means sprayed food plants can be eaten the same day of spraying even though Dipel residues remain. Its oral and dermal toxicity has been tested on a number of mammals including humans, rats, mice, dogs, rabbits, and guinea pigs. No deleterious effects were found. It has also been tested on chickens, pheasants, partridge, and quail, plus rainbow trout (both immatures and matures), blue gills, and Coho salmon. Test results were negative (Anon. 1974).

No phytotoxicity has been reported with Dipel. Some earlier *Bacillus thuringiensis* formulations showed some phytotoxicity; however, this was believed to have resulted from the adjuvants rather than from the active ingredient (Anon. 1974). The virus has been safety tested on selected mammals, birds, fish, and nontarget insects with negative results (Martignoni 1972).

Because it has been well documented that these materials are environmentally safe, the only environmental monitoring planned for this pilot control project is related to the effect of the treatments on other lepidopterans. Contracts are being prepared to be offered to two graduate students, through universities, to examine these effects.

It should be remembered that both of these test materials are naturally occurring and are present at varying levels in most places.

Assessing Foliage Retention

To measure the effect of spray treatments on foliage retention, each sample tree will be rated as to degree of foliage saved. This will be accomplished by visually rating each sample tree prior to tussock moth feeding (late May or early June) and after feeding has been completed in late August. The same person will rate the same plot both times. He will be thoroughly trained in rating defoliation.

A matrix system which is quite sensitive to variation of feeding patterns and proportion of foliage by crown levels will be used.

To account for the variation in crown level feeding characteristic to tussock moth, each tree will be divided into six crown levels of equal length by using a rule with six equal parts. Each crown level will be given a numerical defoliation rating based on the following table:

Table 6.--Crown level ratings

0 - Negligible.	No feeding visible.
1 - Light.	Feeding on new growth only.
2 - Moderate.	Noticeable feeding on new and old growth (less than 50 percent defoliated).
3 - Heavy.	Conspicuous feeding on new and old growth (more than 50 percent defoliated).

Crown levels will be given weights according to their intensity of tussock moth damage. Characteristically the tussock moth defoliates a tree from the top down and by the time the lower crown is defoliated, the impact on that tree is severe and carries more weight than if just the upper crown was fed on. Weights for the tree crown levels will be assigned as 1 (upper one-sixth) to 6 (lower one-sixth). The defoliation index for each tree will be determined by summing the defoliation rating on a crown-by-crown basis (Table 7).

Table 7.--Intensity of defoliation and weighting factors

Crown levels		Negligible 0	Light 1	Moderate 2	Heavy 3	Defoliation index
Upper	1	0	1	2	3	
	2	0	2	4	6	
	3	0	3	6	9	
Mid	4	0	4	8	12	
	5	0	5	10	15	
Lower	6	0	6	12	18	

The numerical value for various combinations of defoliation indexes for each cluster and area can then be tested to see if significant differences occur between mean for various treatments.

Aerial photography will also be used to measure defoliation intensities within the treatment units. False color aerial photography has been shown to be a valuable tool for assessing effects of aerial sprays

applied to suppress forest defoliators by providing estimates of foliage saved in spray blocks as compared with surrounding areas which have not been treated.

Aerial photography of each block will be obtained with Kodak Ektachrome infrared aero film, type 2443, at a scale of 1:15,840 prior to defoliation to establish a base line, and again when the tussock moth larvae have completed their feeding and defoliation is most conspicuous. Aerial photos will be examined in stereo to determine area (acres) protected by the spray application within the designated spray block and adjacent stands. Maps showing areas of foliage protection, degrees of defoliation, and zones of spray drift in relation to the spray blocks will be prepared in accordance with procedures described by Ciesla, et al. (1971).

Data Analyses

Various kinds of data will be produced by the sampling efforts in this pilot test. Some of them are:

1. Estimates of prespray and postspray larval population densities for each tree and treatment block. The estimates of population density will be made from population counts expressed as number of tussock moth larvae-pupae per 1,000 square inches of foliage.

2. Estimation of mortality (population response to the treatments) by ratio estimation; e.g., the survival rate, is given by:

$$r_i = (X_{2i}/Y_{2i})/(X_{1i}/Y_{1i})$$

Where X_{1i} and X_{2i} denote prespray and postspray insect counts and where Y_{1i} and Y_{2i} denote prespray and postspray measurements of square inches of foliage for the ith tree in each treatment area. Survival ratios will be used rather than percentage reduction *per se*, so as to provide a comparison on the same basis as actual survival counts.

3. Visual estimates classifying tree defoliation caused by tussock moth feeding.--Multiple regression techniques will be used to develop formulas to predict defoliation indexes. The two independent variables judged to contribute a major portion of the variance in the regression are (a) prespray population, and (b) condition of the tree prior to feeding or prespray tree index. Multiple regression formulas will be developed from control areas where the infestation is allowed to run its natural course. One other variable which might be entered is the level of natural virus in the area. If we are able to measure this for the different areas from our virus evaluation, we will enter it as the third independent variable.

The regression formula would appear as:

Y = BO + X1 (prespray population) + X2 (prespray defoliation index)
When Y = predicted defoliation index
BO = regression intercept
X1 = regression coefficient for prespray population
X2 = regression coefficient for prespray defoliation index

A paired T test can then be used to test significant difference between the actual and predicted defoliation indexes. If the means are significant and the predicted index mean is higher than the actual, then foliage was saved by control efforts.

4. Estimates describing a range of deposited dosage for each sample tree for each microbial treatment.--The relationships between insect mortality and spray deposits will be described by regression techniques.

5. Visual estimates classifying tree defoliation caused by tussock moth feeding:

a. Aerial photographs

b. Classification by undamaged, partly damaged, and severely damaged categories.

6. Variations in parasite populations related to the different sampling periods.

INFORM AND INVOLVE

Publicity and public involvement will be coordinated by the I&I Officer.

An environmental analysis will be made to appraise the effects of the project on all segments of the environment. This report will be available for public review.

Appropriate news releases and public meetings will be held in key areas. Groups and individuals having special interest in the project will be met with to answer project related questions.

COOPERATION

This pilot control project will be a cooperative effort between the Division of State and Private Forestry, Region 1; Aerial Application Project (PNW-2208); Forest Insect Disease Project (PNW-2203); Missoula Equipment Development Center (MEDC); and Division of Timber Management, Region 6. This study plan was prepared jointly by members from each of these groups. Publication of the results of this field test will be under the joint authorship of the appropriate cooperators.

Specific areas of responsibility follow:[2/]

1. Design, planning, and review of test. Region 1, Division of State and Private Forestry; Nezperce and Idaho Panhandle National Forests; PNW-2208; PNW-2203; and Division of Timber Management, Region 6.

2. Development and conduct of inform and involve program. Nezperce and Idaho Panhandle National Forests and Division of I&E, Region 1.

3. Preparation of appropriate environmental analyses. Region 1, Division of State and Private Forestry, Nezperce and Idaho Panhandle National Forests, PNW-2208, and PNW-2203.

4. Preliminary work on mixing and application techniques. PNW-2208 and MEDC.

5. Virus contracting and materials quality control. PNW-2203; Division of State and Private Forestry, Region 1; and Division of Timber Management, Region 6.

6. Contracting of aircraft. Region 1, Air Operations and Division of State and Private Forestry.

7. Employment of temporary personnel and selection of field laboratory site. Nezperce and Idaho Panhandle National Forests.

8. Selection of spray blocks and heliport location. Region 1, Division of State and Private Forestry; PNW-2203 and 2208; MEDC; Nezperce and Idaho Panhandle National Forests.

9. Calibration, determination of spray atomization, swath width, etc. PNW-2208 and MEDC.

10. Mixing, loading, and ground handling. MEDC and PNW-2208.

11. Spray deposit assessment. PNW-2208.

12. Meteorological support. U.S. Weather Service and MEDC.

13. Laboratory operations. Division of State and Private Forestry, Region 1; and PNW-2203.

14. Aerial photography and photo interpretation. Region 1, Division of Engineering and Division of State and Private Forestry.

2/ If Region 1 test sites prove unsuitable and alternative suitable test sites are located in Region 6, then Region 6 Timber Management personnel will have involvement in all aspects of this test.

15. Training and supervision of field crews. Region 1, Division of State and Private Forestry; PNW-2208; PNW-2203; and MEDC.

16. Analysis of data and reporting of results. Region 1, Division of State and Private Forestry; PNW-2208; PNW-2203; and MEDC.

In conjunction with the pilot control project, MEDC and Dugway Proving Grounds (Department of Defense) will be evaluating meteorological equipment. This will include placing additional spray deposit assessment cards. The equipment will not be tested on all plots. No supporting funds or personnel will be needed from the pilot control project for this satellite study.

ORGANIZATION

The organization chart for this project is shown in Figure 3.

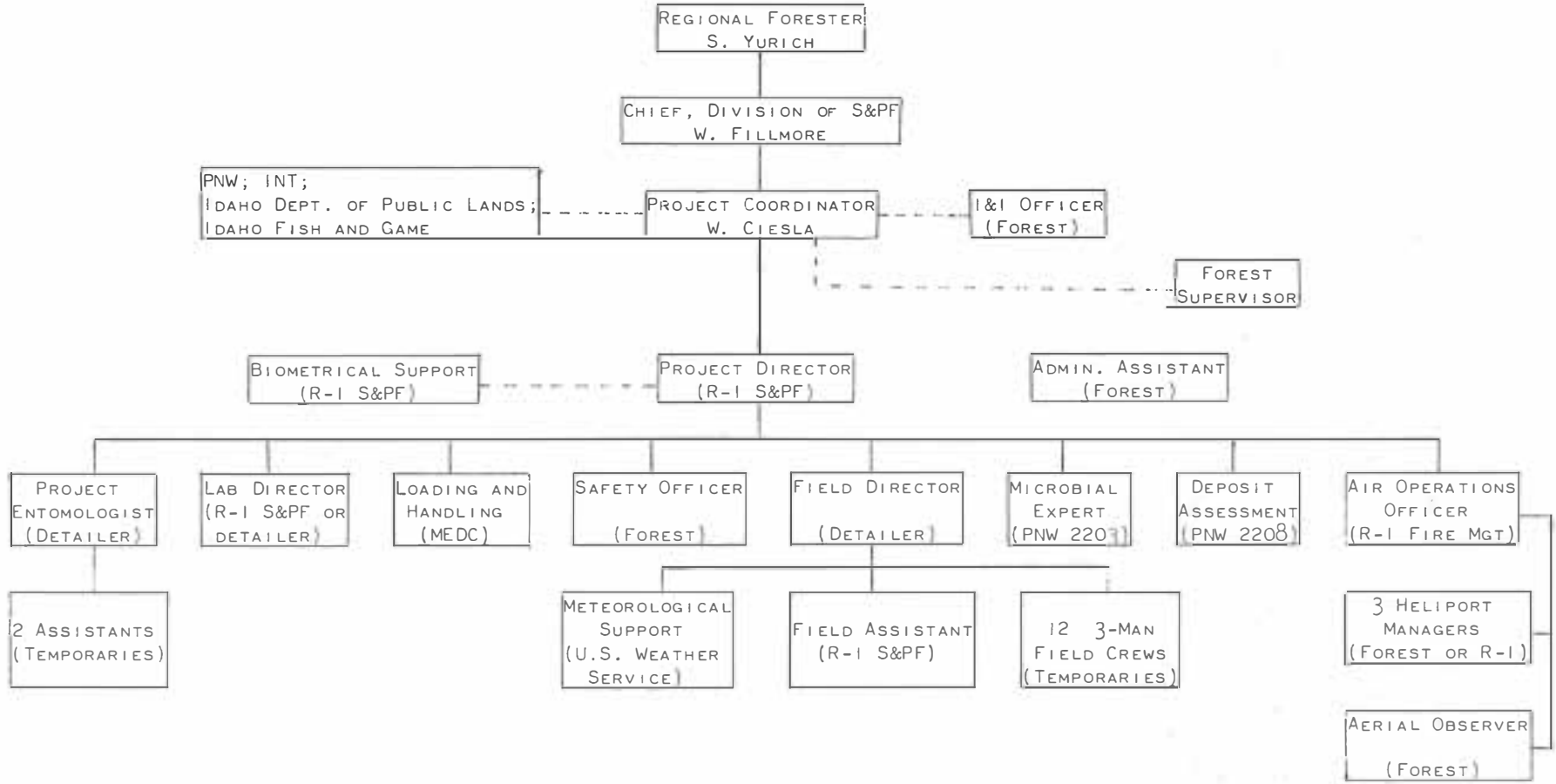


Figure 3.--Organizational chart for 1974 pilot control project for Douglas-fir tussock moth, Region 1. This organization will be duplicated at the Coeur d'Alene Mountain area and the Lookout Butte area.

FINANCIAL PLAN FOR 1974 PILOT CONTROL PROJECT FOR DOUGLAS-FIR TUSSOCK MOTH, REGION 1

Item	FY 1974	Rate	FY 1975	Rate
Materials				
Dipel	$ 80,000	20,000 lbs. @ $4/lb.		
Virus*	85,000	10,000 lbs. @ $8.50/lb.		
Carrier (molasses)	22,500	15,000 gal. @ $1.50/gal.		
Additives (dye, buffer, sunscreen)	12,000			
Aircraft**				
Helicopters	105,000	30,000 acres @ $3.50		
Chase planes	800			
Salaries				
Field crews	25,920	72 men @ $3/hr. for 15 days	$ 60,480	72 men @ $3/hr for 35 days
MEDC	11,880	9 men @ $88/day for 20 days		
Overtime	23,200		25,200	
Vehicle rental				
Pickup trucks	10,560	40 pickups @ $440/day for 24 days	18,040	40 pickups @ $440/day for 41 days
Gas and oil for vehicles	2,000		3,000	
3,000-gallon tankers	5,000	2 trucks @ $175/day for 14 days		
Per diem	8,000	16 men @ $20/day for 25 days	12,800	16 men @ $20/day for 40 days

*Financing of virus has already been covered; $44,000 worth of virus is now being contracted for, the remainder is on hand from a 1972 contract.

**May require some financing in 1975, depending on weather and larval development.

FINANCIAL PLAN FOR 1974 PILOT CONTROL PROJECT FOR DOUGLAS-FIR TUSSOCK MOTH, REGION 1, con.

Item	FY 1974	Rate	FY 1975	Rate
Equipment and supplies	$ 10,700		$ 1,800	
I&I	3,000			
Deposit assessments	3,500		8,100	
Freight				
Carrier	4,500			
Mixing unit	500			
Planning, training, and related activities	3,000			
Environmental monitoring	3,000		4,500	
Computer time			1,000	
Laboratory and coolers	400		600	
Aerial photography	1,500		1,500	
	$419,960		$137,980	

Total	$557,940
Virus costs	-85,000
Financing needed	$472,940

REFERENCES CITED

Anonymous, 1973. USDA Forest Service Environmental Statement, Douglas-fir tussock moth pest management plan, Oregon and Washington, 1973, USDA Forest Service, Pacific Northwest Region, Portland, Oregon.

Anonymous, 1974. Environmental analysis, Douglas-fir tussock moth, microbial insecticide pilot tests, appendix section D, USDA Forest Service, Northern Region, Missoula, Montana.

Ciesla, W. M., L. E. Drake, and D. H. Wilmore, 1971. Color photos, aerial sprays and the forest tent caterpillar. Photogrammetric Engr. 37: 867-873.

Martignoni, M., 1970. A production control procedure for nucleopolyhedrosis virus preparations. USDA Forest Service, Pac. NW Forest and Range Expt. Sta., Corvallis, Oregon (progress report).

Martignoni, Mauro E., 1972. Production, safety, evaluation, and infectivity titer assay of a nucleopolyhedrosis virus preparation for field application. USDA Forest Service, Pac. NW Forest and Range Expt. Sta., Corvallis, Oregon (progress report).

Snedecor, George W., 1956. Statistical methods. The Iowa State College Press, Ames, Iowa, pp. 512-515.

Stelzer, Milton J., and John Neisses, 1973. Study plan aerial applications of a nucleopolyhedrosis virus and *Bacillus thuringiensis* against the Douglas-fir tussock moth: population survival rates, initial residual levels of infection, spray deposits-mortality relationships. USDA Forest Service, Pac. NW Forest and Range Expt. Sta., Portland, Oregon (unpub. report).

Thompson, C. G., and G. P. Markin, 1973. Preliminary results of microbial spray tests against the Douglas-fir tussock moth in 1973. USDA Forest Service, Pac. NW Forest and Range Expt. Sta., Portland, Oregon (unpub. report).

Tunnock, Scott, 1963. Trends of Douglas-fir tussock moth infestations in northern Idaho and Colbert, Washington. USDA Forest Service, Div. State and Priv. Forestry, Missoula, Montana (unpub. report).

Tunnock, Scott, 1973. Douglas-fir tussock moth in the Northern Region--a cartographic history of outbreaks from 1928 to 1973. USDA Forest Service, Northern Region, Div. State and Priv. Forestry, report 73-27.

Wickman, B. E., R. R. Mason, and C. G. Thompson, 1973. Major outbreaks of the Douglas-fir tussock moth in Oregon and Washington. USDA Forest Service, general tech. report PNW-5, 18 pp.

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE
Corvallis, Oregon 97331

APPENDIX I

REPLY TO: 4500 Forest Insect Research December 3, 1973

SUBJECT: Clean-up of nucleopolyhedrosis virus for the Douglas-fir tussock moth pilot test

TO: William M. Ciesla, Coordinator, Environmental Services
Division of State and Private Forestry

It is my understanding that you intend to use the tussock moth virus that is currently in stock at the PNW Station for the 1973 pilot control test. This poses another problem in addition to the mixing and application problems we discussed in Portland November 26 & 27. This virus stock is the "dirty" virus that I mentioned in Portland. We screened (filtered) the quantities of virus that were used for the 1972 field experiments to reduce the number of large extraneous particles (body parts and hairs). You will have to do the same for the ca. 7000-acre equivalents of virus you intend to use for the pilot test.

I have discussed this problem with Dr. Mauro Martignoni and Paul Iwal, who did the actual screening for the field experiment. We came up with the following screening procedure. The filtering process has to be done in a liquid suspension. It can't be done in a dry state.

A couple of days before a proposed application date, the virus, in the freeze-dried state, can be suspended in a 0.02 molar solution of Trizma-pH 7.1 buffer. Enough virus should be mixed to allow for about a 20% loss in polyhedra count during the filtration process. We suspended 10-acre equivalents ($1.0X10^{11}$ polyhedra) of virus in 150 ml of buffer, but we would recommend that you use a proportionately larger quantity of buffer since our suspensions were quite thick. Ten to 15 gallons of buffer per 1,000-acre equivalents would probably be a better ratio.

The Trizma-pH 7.1 buffer can be obtained from Sigma Chemical Company:

Sigma Chemical Co.
P.O. Box 14508
St. Louis, MO
Ph: 314-771-5750

You should specify reagent grade quality with an average molecular weight of 154.7. The pH should be checked, after the buffer solution has been mixed, but before the freeze-dried virus is added. The pH can be checked with a conventional portable pH meter. However, the

reading should not be taken immediately after putting the electrodes in the solution. One or two minutes should be allowed for the hydrogen ions to react with the probes. Triz buffer does not react well with pH paper, so a meter has to be used to check the pH.

A small capacity mixing tank, equipped with some type of recirculation, should be used for this process. The mixing equipment will have to be equipped with some type of filtering capability which allows for quick exchange and cleaning of the filtering screens. Three mesh sizes are recommended for the cleaning process; 50, 100, and 150 (maybe even a 200) mesh screens. The filtering process starts with the coarse mesh and progresses to the finer mesh screens as the larger hairs and body parts are strained from the virus suspension.

A polyhedra count will have to be made of the final screened virus suspension. Based on such a count, the proper amount of the screened virus suspension can then be added to the virus spray formulation to give dose of $1X10^{11}$ polyhedra per acre. Someone from Hank Thompson's project should work with someone from R-1, probably Mark McGregor, to familiarize them with the counting procedure and other bioassay details.

Further details of this clean-up problem and the requirements and design of the small mixing tank can be further discussed during the Missoula meeting, December 11, at which time we can get some input from MEDC. I feel that this whole clean-up process should be part of the study plan because "dirty" virus of this type might be encountered in the future, even though procurement contracts may specify the required quality.

If you have any questions, please feel free to give me a call.

JOHN NEISESS
Chemist

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE
3200 Jefferson Way
Corvallis, Oregon 97331

REPLY TO: 4500 Forest Insect Research December 14, 1973

SUBJECT: Cleaning of nucleopolyhedrosis virus preparation

TO: William Ciesla

I have some additional information concerning the cleaning of the virus.

1. The average weight (before screening) of the 1972-1973 nutrilite produced virus for the 10^{11} polyhedra/acre dose is 7.226 grams. A 1,000-acre dose would be 7.226 kilograms.

2. I was in error in the recommended ratio of virus to buffer solution. The correct ratio of virus to buffer is 1:10 (w/v). Thus, 72.26 liters of buffer solution (19.1 gallons) are needed for suspending 1,000-acre doses of virus prior to screening. Any ratio less than 1:10 (w/v) results in suspensions which are unsuitable for fast flow through the fine mesh screens. Therefore, the 10 to 15 gallons per 1,000-acre dose I suggested in the December 3 memo would not provide a sufficiently dilute suspension.

If you need any other information for the pilot test, either Milt Stelzer or I should be able to supply it. Sorry I wasn't able to be in Missoula for the meetings.

JOHN NEISESS
Chemist

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE

REPLY TO: 4500 Forest Insect Research January 17, 1974

SUBJECT: 1974 Microbial Pilot Test-formulations

TO: Tony Jasumback, MEDC

I am enclosing the information concerning the cleanup of the virus and technical information dealing with Santoquin - one of the stabilizers added to the CIB, as we discussed January 10, 1974. Dick and I were very happy to hear that you were going to be involved in the pilot project.

I got to thinking about the possible use of the large helicopters. If the larger aircraft are to be used, a larger nozzle tip size would be used than what we used with the Bell 47G. We have conducted tests with a 25% molasses-Dipel (B.t) formulation using T8010 tips and flying at 90 m.p.h. The resulting VMDs were 266 and 321 microns for 60 and 40 psi, respectively. We also used a molasses-Thuricide (B.t.) formulation which had about the same viscosity, but a lower surface tension as the Dipel formulation. The VMDs for this formulation were 199 and 231 microns at 60 and 40 psi. We did not use any screens in the application equipment. If a Bell 205 or 206 helicopter were used for the pilot tests the plugging problems that we encountered would essentially be eliminated by simply removing all the screens from the equipment. The formulations would still have to be thoroughly filtered or screened while mixing and loading.

We will be looking forward to hearing from you on your progress with the other mixing and handling problems.

JOHN NEISESS
Chemist